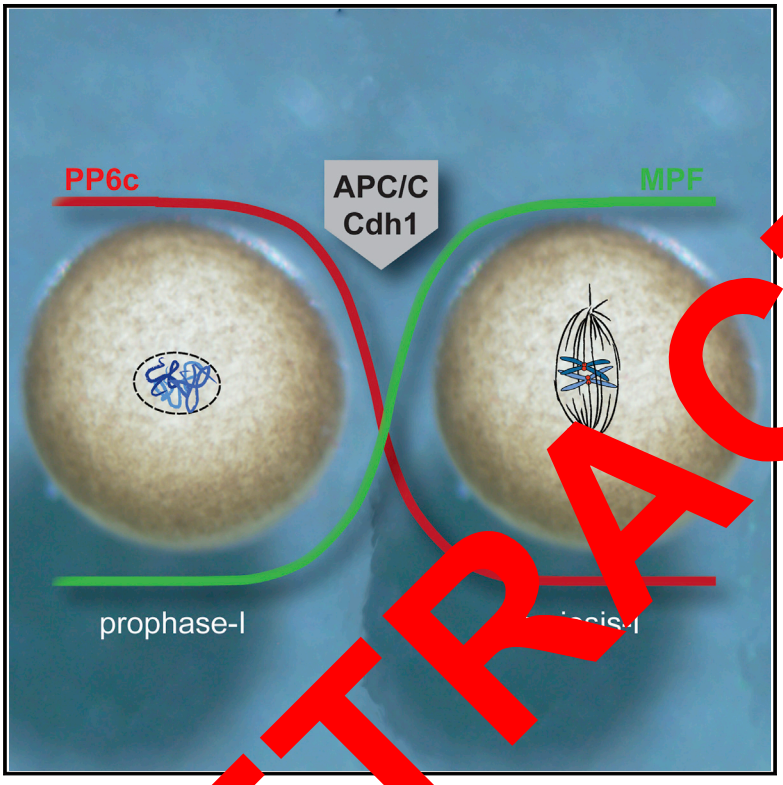
Short Article

Developmental Cell

The Anaphase-Promoting Complex/Cyclosome Is Essential for Entry into Meiotic M-Phase

Graphical Abstract



Highlights

- MPF activa[illegible] immature *Xenopus* oocytes requires the ligas[illegible]tivity [illegible]APC/C^{Cdh1}
- [illegible]e cata[illegible]ic subuni[illegible]t of protein phosphatase PP6 is the re[illegible] APC/C^{Cdh1} substrate
- PP6c d[illegible]ruction enables Aurora A activation, resulting in synthesis of M-phase proteins

Authors

Saurav Malhotra, Palakkad K[illegible]nanu[illegible]Vinod, Jörg Ma[illegible]eld, C[illegible]af Ste[illegible]nn, Thoma[illegible]M[illegible]er

Correspon[illegible]
tho[illegible]s.u.mayer@uni-konstanz.de

In Brief

The ubiquitin ligase APC/C is required for e[illegible]ting M-phase during cell division. Malhotra et al. show that APC/C is also critical for entry into M-phase of meiosis I upon progesterone stimulation of immature *Xenopus* oocytes. The catalytic subunit of protein phosphatase 6 is the relevant APC/C^{Cdh1} substrate for meiotic resumption.

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The Anaphase-Promoting Complex/Cyclosome Is Essential for Entry into Meiotic M-Phase

Saurav Malhotra,[1] Palakkad Krishnanunni Vinod,[2,5] Jörg Mansfeld,[3] Olaf Stemmann,[4] and Thomas U. Mayer[1,*]
[1]Department of Molecular Genetics, Konstanz Research School Chemical Biology (KoRS-CB), University of Konstanz, Universitätsstrasse 10, 78457 Konstanz, Germany
[2]Department of Biochemistry, OCISB, University of Oxford, South Parks Road, Oxford OX1 3QU, UK
[3]Cell Cycle, Biotechnology Center, TU Dresden, 01307 Dresden, Germany
[4]Department of Genetics, University of Bayreuth, Universitätsstrasse 30, 95440 Bayreuth, Germany
[5]Present address: CCNSB, International Institute of Information Technology, Hyderabad 500032, India
*Correspondence: thomas.u.mayer@uni-konstanz.de
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SUMMARY

Vertebrate immature oocytes are arrested at prophase of meiosis I (MI). Hormonal stimulation breaks this prophase-I arrest and induces re-entry into MI. The mechanism underlying meiotic resumption remains largely elusive. Here, we demonstrate that the anaphase-promoting complex/cyclosome (APC/C) in complex with Cdh1 has an unexpected function in meiosis in that it is essential for meiotic resumption. We identify the catalytic subunit of protein phosphatase 6 (PP6c) as a critical substrate whose APC/C^{Cdh1}-mediated destruction is a prerequisite for the re-entry of immature *Xenopus laevis* oocytes into MI. Prevention of PP6c destruction impairs activating autophosphorylation of Aurora A, a cell-cycle kinase critical for meiotic translation. Restoring meiotic translation rescues the meiotic resumption defect of Cdh1-depleted oocytes. Thus, our studies discover that the essential function of the APC/C in triggering cell-cycle transitions is not limited to M-phase exit but also applies to entry into meiotic M-phase. We identify a crucial APC/C-PP6c-Aurora A axis for the resumption of female meiosis.

INTRODUCTION

Following homologous recombination between paternal and maternal chromosomes, vertebrate immature oocytes arrest at prophase of meiosis I (MI). This prophase-I arrest, which in mammals can last up to decades, is characterized by the lack of activity of the maturating promoting factor (MPF) composed of cyclin-dependent kinase 1 (Cdk1) and its regulatory subunit cyclin B. In immature *Xenopus* oocytes, Cdk1 and cyclin B2 form a pre-MPF complex where Cdk1 is kept inactive by inhibitory phosphorylations at threonine 14 (T14) and tyrosine 15 (Y15) (Dunphy and Newport, 1989; Gautier and Maller, 1991). Progesterone (PG) triggers a signaling cascade resulting in the activation of MPF and, consequently, meiotic resumption. After completing the first meiotic division, oocytes arrest again at metaphase MII where they await fertilization. Essential for the MII arrest is Emi2/XErp1, an evolutionarily conserved inhibitor of the APC/C (Schmidt et al., 2005; Shoji et al., 2006). Fertilization triggers the destruction of XErp1 resulting in the activation of the APC/C, which then induces exit from meiotic M-phase by targeting securin and cyclin B for destruction (Liu and Maller, 2005; Rauh et al., 2005). Thus, consistent with its essential function in triggering exit from mitotic M-phase, the ability of mature oocytes to exit meiosis critically depends on the activity of the APC/C. However, little is known about the function of the APC/C in maintaining the prophase-I arrest of immature *Xenopus* oocytes and their hormone-induced entry into meiosis. Here, we show that the ligase activity of the APC/C is completely dispensable for a robust prophase-I arrest. Intriguingly, however, entry into meiotic M-phase strictly depends on the activity of the APC/C in complex with its co-activator Cdh1. The search for the critical substrate identified the catalytic (c) subunit of protein phosphatase 6 (PP6). In vitro, ubiquitylation of PP6c is catalyzed by APC/C^{Cdh1} but not APC/C^{Cdc20}. Interfering with PP6c destruction in oocytes by depleting Cdh1 or expressing a destruction box mutant PP6c blocks MI entry. As a potential substrate of PP6c we identify Aurora A, which plays an important role in meiotic resumption by inducing the translation of M-phase-promoting factors. Our studies identify that the APC/C, in addition to its well-established function in triggering exit from M-phase, is essential for entry into M-phase of MI, and discovered a novel pathway essential for progesterone-induced meiotic resumption.

RESULTS

APC/C in Complex with Cdh1 Is Essential for Meiotic Resumption

To investigate whether the APC/C is critical for the prophase-I arrest, fully grown immature *Xenopus laevis* oocytes, termed stage-VI oocytes, were surgically removed and injected with mRNA encoding Flag-tagged C-terminal (^{CT}) fragment of the APC/C inhibitor XErp1 (Nishiyama et al., 2007; Schmidt et al., 2005; Tang et al., 2008). The APC/C inhibitory activity of Flag-XErp1^{CT} was confirmed by its ability to block the calcium-induced degradation of cyclin B in extracts of metaphase-II arrested oocytes (Figure S1). Immature stage-VI oocytes expressing Flag-XErp1^{CT} maintained the prophase-I arrest as

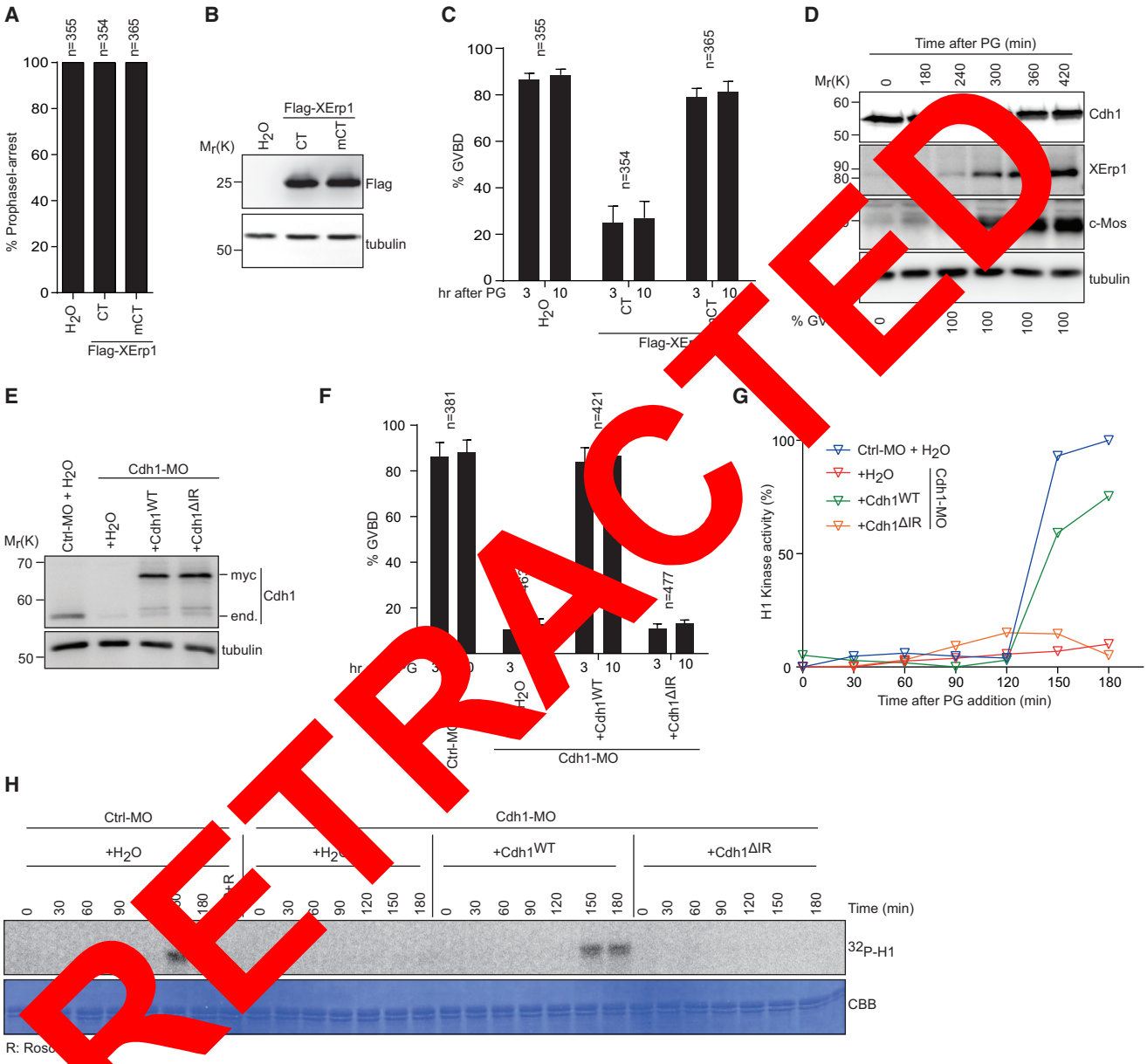

Figure 1. APC/C^{Cdh1} Activity Is Essential for Resumption of Vertebrate Female Meiosis

(A) Immature *Xenopus* oocytes were injected with H_2O or mRNA encoding Flag-XErp1^{CT}, or Flag-XErp1^{mCT}. Fourteen hours after injection, the percentage of oocytes that maintained the prophase-I arrest was quantified. n indicates the total number of oocytes analyzed in three independent experiments. The APC/C inhibitory activity of Flag-XErp1^{CT} but not Flag-XErp1^{mCT} was confirmed in extract of metaphase-II arrested eggs (see Figure S1).

(B) Oocytes from (A) were lysed and immunoblotted for Flag-tag. α-Tubulin served as loading control.

(C) Oocytes from (A) were treated with progesterone (PG) for 3 and 10 hr and then quantified for their ability to undergo GVBD. Error bars indicate SD.

(D) At indicated time points after PG stimulation, oocytes were lysed and immunoblotted for Cdh1. XErp1 and c-Mos served as markers for meiotic progression.

(E) Stage-VI oocytes were injected with Ctrl-MOs or Cdh1-MOs and H_2O or mRNA encoding myc-tagged human Cdh1^{WT} or Cdh1$^{\Delta IR}$. Fourteen hours after injection, oocytes were lysed and immunoblotted for Cdh1.

(F) Three and ten hours after PG treatment of oocytes from (E), their ability to undergo GVBD was quantified.

(G) Quantification of autoradiogram from (H) using the "measure tool" of ImageJ.

(H) Oocytes from (E) were lysed at indicated time points after PG stimulation and lysates supplemented with histone H1 and [γ-^{32}P]ATP. [γ-^{32}P] incorporation was analyzed by autoradiography. The Cdk1 inhibitor roscovitine (R) was used at 10 μM.

efficiently as control-injected (H_2O) ones, indicating that the ligase activity of the APC/C is dispensable for the prophase-I arrest (Figures 1A and 1B). To test whether the APC/C is required for PG-induced entry into MI, control and Flag-XErp1^{CT} injected oocytes were treated with PG, and the percentage of oocytes undergoing meiotic resumption was quantified based on the

appearance of the germinal vesicle (GV) characteristic white spot at the animal pole and its subsequent breakdown (GVBD). Notably, while 86.6% ± 4.4% of control-injected (H_2O) oocytes entered MI within 3 hr after PG treatment, only 27% ± 12% of Flag-XErp1^{CT}-injected oocytes underwent GVBD within even 10 hr after PG exposure (Figures 1B and 1C). To confirm that the failure in meiotic resumption was due to APC/C inhibition, we injected a mutant version of Flag-XErp1^{CT} (XErp1^{mCT}) unable to inhibit the APC/C (Figure S1). 79% ± 6.5% of these oocytes entered MI within 3 hr after PG exposure (Figure 1C). Consistent with the idea that the ligase activity of the APC/C is critical for MI entry, injection of glutathione S-transferase (GST)-tagged TUBEs—"Tandem Ubiquitin Binding Entities" that bind with high affinity to polyubiquitylated proteins and protect them from proteasomal destruction (Hjerpe et al., 2009)—efficiently prevented PG-induced meiotic resumption (Figure S1). We conclude that *Xenopus* oocytes depend on APC/C activity not for the maintenance of the prophase-I arrest but for entry into the first meiotic division.

Immature oocytes arrested at prophase-I are characterized by low Cdk1 activity. Binding of Cdc20 to the APC/C requires the phosphorylation of core APC/C subunits by Cdk1, whereas Cdh1 binding to the APC/C is inhibited by phosphorylation of Cdh1 by Cdk1 (Kraft et al., 2003, 2005; Kramer et al., 2000; Rudner and Murray, 2000; Vodermaier et al., 2003; Zachariae et al., 1998). We therefore reasoned that Cdh1 is the relevant APC/C co-activator. However, probably due to low expression levels, initial studies failed to detect Cdh1 in *Xenopus* oocytes (Lorca et al., 1998). In line with recent reports (Pan et al., 2004; Zhou et al., 2002), our analysis revealed that Cdh1 is indeed expressed in immature oocytes and by using c- and XErp1 as markers for meiotic progression we could demonstrate that Cdh1 levels remained constant until MII (Figure 1D). Next, we depleted Cdh1 by injecting morpholino oligonucleotides (MOs) targeting Cdh1 mRNA into stage-VI oocytes. Immunoblotting confirmed that injection of Cdh1-MOs, but not scrambled control (Ctrl) MOs, resulted in efficient Cdh1 depletion (Figure 1E). Ctrl-injected oocytes remained in prophase-I arrest (Figure S1) and efficiently 86.3% underwent GVBD within 3 hr after PG treatment (Figure 1F). Cdh1-MOs injected oocytes were also fully capable of maintaining the prophase-I arrest (Figure S1), but only 12.6% ± 2.5% of these oocytes underwent meiotic resumption within 10 hr after PG treatment (Figure 1F). Co-injection of mRNA encoding myc-tagged wild-type (WT) human Cdh1 together with Cdh1-MOs completely rescued the meiotic resumption defect (Figures 1E and 1F), confirming that the failure to enter MI was specific for Cdh1 depletion. Human Cdh1 lacking the C-terminal IR motif (ΔIR), essential for binding to the APC/C (Vodermaier et al., 2003), failed to rescue the defect of Cdh1-depleted oocytes to enter MI (Figures 1E and 1F). To quantify entry into MI by an independent assay, we determined Cdk1 kinase activity in extracts of PG-treated oocytes using histone H1 as substrate and [γ-^{32}P]ATP. In full agreement with our phenotypic analysis, Cdh1 depletion prevented PG-induced Cdk1 activation, and this defect was rescued by the expression of human WT Cdh1 but not the ΔIR mutant (Figures 1G and 1H). We conclude that the function of Cdh1 as APC/C co-activator is essential for PG-triggered MPF activation in immature *Xenopus* oocytes.

Meiotic Resumption Depends on Destruction of the Catalytic Subunit of PP6

An immediate response of the PG signaling cascade is the inactivation of protein kinase A (PKA), an event which is necessary and sufficient to trigger meiotic resumption (Duckworth et al., 2002; Huchon et al., 1981; Maller and Krebs, 1977; Sadler and Maller, 1981). To test whether Cdh1 depletion prevents PKA inactivation, we quantified PKA activity in extracts of Cdh1- and Ctrl-MOs injected oocytes using the PKA-specific substrate Kemptide and [γ-^{32}P]ATP. Extracts of mature oocytes displayed maximal Kemptide phosphorylation, and this activity was mediated by PKA and was efficiently suppressed by the PKA-specific inhibitor H89 (Figure S1). Importantly, PKA inactivation upon PG stimulation occurred with similar kinetics in oocytes injected with Cdh1-MOs or Ctrl-MOs (Figure 2A), indicating that loss of Cdh1 does not interfere with PKA inactivation by an upstream event. Based on these findings, we speculated that an inhibitor of MI entry exists whose APC/C^{Cdh1}-mediated destruction is critical for meiotic resumption. To identify this potential factor, prophase-I arrested oocytes (Ctrl) and PG-treated oocytes undergoing GVBD were lysed and immunoblotted for the known Cdk1 antagonist Myt1 and a panel of protein phosphatases since these have been recognized as key opponents of M-phase entry (Lorca and Castro, 2013; Mochida and Hunt, 2012). Wee1 was excluded from the analyses because it is not expressed in immature *Xenopus* oocytes (Nakajo et al., 2000). Immunoblotting for inhibitory Cdk1 phosphorylations (pT14/pY15) and c-Mos confirmed the correct cell-cycle stage of the extracts (Figure 2B). Of the tested candidates, only the catalytic (c) subunit of PP6 displayed the anticipated behavior in that it was abundant in prophase-I extract, but hardly detectable in GVBD extract (Figure 2B). PP6 is a trimeric type-2A serine/threonine phosphatase composed of a catalytic (c), a SAP (SIT4 phosphatase-associated protein) regulatory subunit, and an ankyrin repeat domain subunit (Stefansson et al., 2008). Initial studies identified PP6 as a functional homolog of the cell-cycle phosphatases Sit4p and ppe1 of budding and fission yeast, respectively (Bastians and Ponstingl, 1996). Next, we investigated when exactly during oocyte maturation PP6c disappears. For this and all subsequent experiments, we used a commercially available antibody that was specific for PP6c (Figure S2). Immunoblot analyses revealed that PP6c was present in prophase-I arrested oocytes, but barely detectable at 180 min after PG stimulation (Figure 2C). Quantification of the percentage of oocytes undergoing GVBD and immunoblotting for c-Mos revealed that the disappearance of PP6c coincided with GVBD. Subsequently, PP6c re-accumulated as oocytes approached the metaphase-II arrest (Figure 2C). To test whether the decline in PP6c levels was mediated by APC/C^{Cdh1}, Ctrl- and Cdh1-MOs injected oocytes were immunoblotted for PP6c. Indeed, PP6c remained completely stable in PG-treated oocytes depleted of Cdh1 (Figure 2D). The stabilization of PP6c could be cause or consequence of Cdh1-depleted oocytes not being able to enter MI. To distinguish between these two possibilities, we analyzed whether PP6c degradation is a prerequisite for MI entry. Sequence analysis of PP6c identified conserved amino acid residues matching the extended destruction box consensus motif $Rx_2Lx_{3-4}N$ (Figure 2E). Injection of mRNA encoding myc-tagged PP6c with a mutated destruction

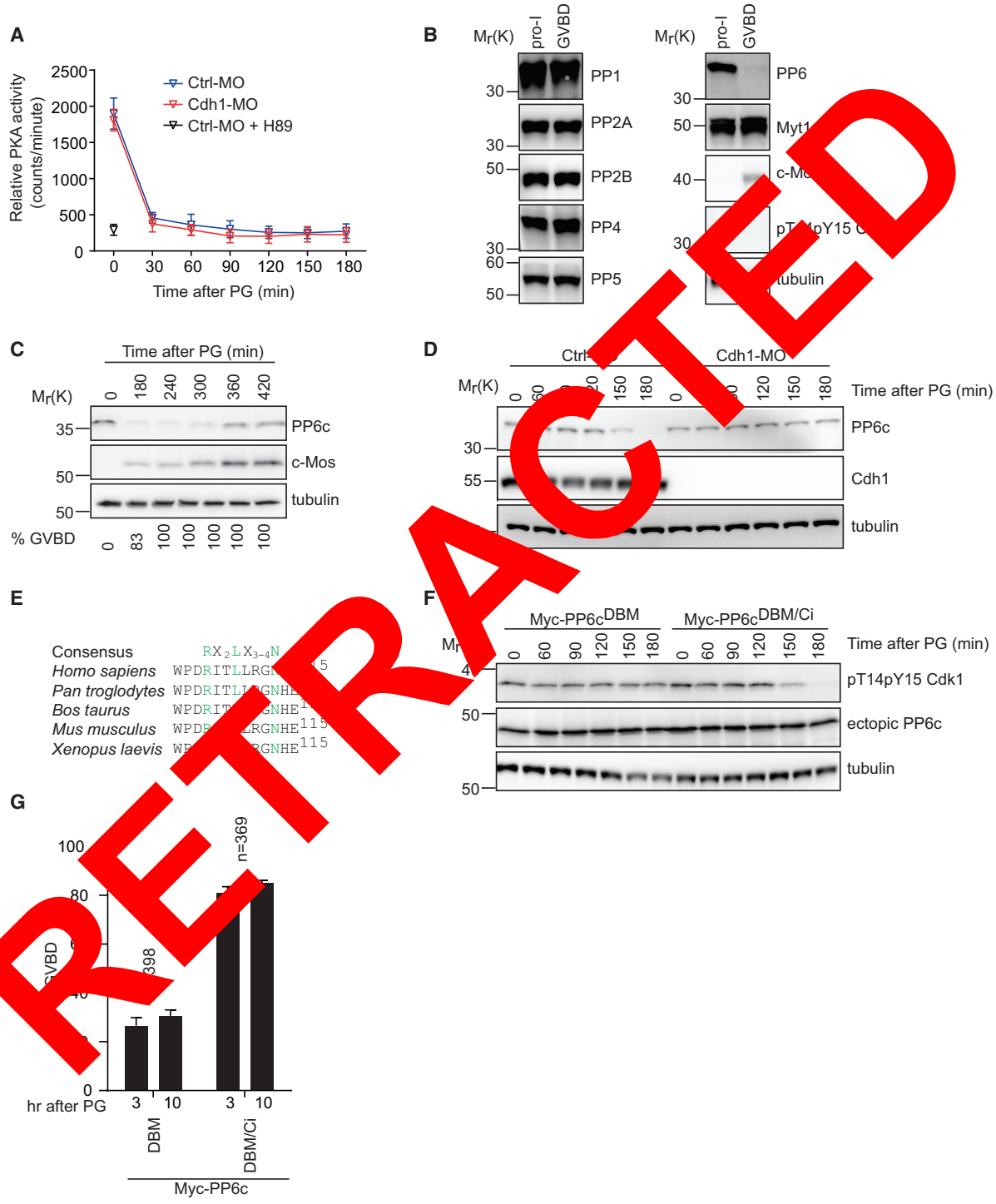

Figure 2. PP6c Destruction Is a Prerequisite for Meiotic Resumption

(A) Immature oocytes injected with Ctrl-MOs or Cdh1-MOs were lysed at indicated time points after PG stimulation. Lysates were supplemented with the PKA substrate Kemptide and [γ-^{32}P]ATP. [γ-^{32}P] incorporation was quantified by scintillation counting. 100 μM PKA-inhibitor H89 was added to lysate of Ctrl-MOs oocytes not treated with PG. Error bars indicate SD.

(B) Untreated immature oocytes (pro-I) and oocytes treated with PG for 3 hr (GVBD) were lysed and immunoblotted for the indicated proteins. c-Mos and inhibitory Cdk1 phosphorylations (pT14pY15) served as markers for meiotic progression.

(C) Prophase-I arrested immature oocytes were treated with PG, and at indicated time points thereafter the percentage of oocytes undergoing GVBD was quantified. Lysates were prepared and immunoblotted for PP6c, c-Mos, and α-tubulin.

(D) At indicated time points after PG treatment, Ctrl-MOs or Cdh1-MOs injected oocytes were lysed and immunoblotted.

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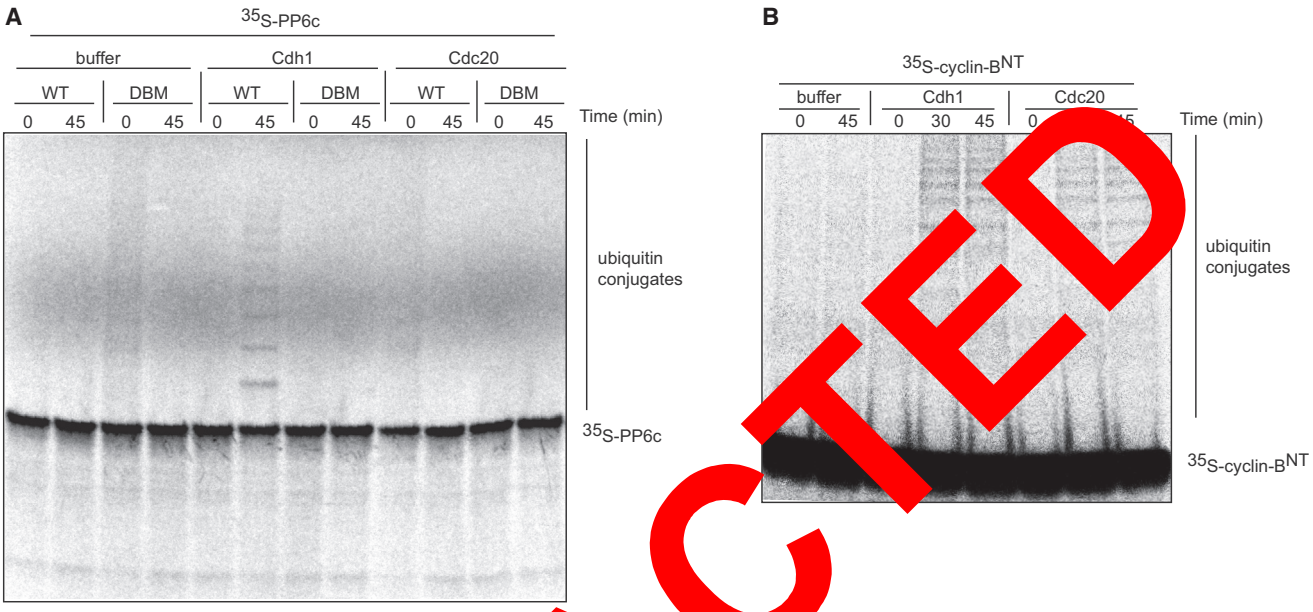

Figure 3. PP6c Is a Substrate of APC/C^{Cdh1}

(A) In vitro ubiquitylation assays using ^{35}S-labeled in vitro translate[d] wild-type [and] destruction box mutant (DBM) ^{35}S-PP6c as substrate. The reactions were supplemented with recombinant E1, E2, ubiquitin, immunopurified A[PC/C] and [Cdh1]-C[ycB], His-Cdc20, or control buffer. At indicated times, samples were analyzed by autoradiography.

(B) Assay as in (A) except that an N-terminal fragm[ent] of cyclin B (^{35}S-cyclin-B^{NT}) was used as substrate.

box (AxxAxxxN, DBM) revealed that [PP6c] remained stable upon PG treatment, wh[ereas] ectopic [wild-type] PP6c^{WT}, like the endogenous protein, was d[egra]ded in respo[nse] to PG stimulation (Figures 2F and S2), [thus] confirming the identified motif as a bona fide destruction box. Importantly, only 30.6% ± 4% of the oocytes expressing myc-PP6c^{DBM} entered MI within 10 hr after PG stimulation (Figure 2G). Immunoblotting for inhibitory Cdk1 phosphorylations confirmed the failure of these oocytes to enter MI (Figure 2F). In contrast, 81% ± 4% of oocytes expressing catalytically inactive version of stable PP6c (myc-PP6c[Ci]) matured (Figures 2F and 2G). We conclude that inactivation of PP6c by means of degradation is critical for the ability of oocytes to resume meiosis in response to PG stimulation.

APC/C^{Cdh1} Ubiquitylates PP6c

To test whether PP6c is a direct substrate of APC/C^{Cdh1}, we performed ubiquitylation assays using in vitro translated ^{35}S-labeled PP6c as substrate. Addition of recombinant Cdh1 markedly increased the ability of immunopurified APC/C to ubiquitylate WT [^{35}S]PP6c (Figure 3A). In contrast, recombinant Cdc20 did not significantly support polyubiquitylation of WT [^{35}S]PP6c (Figure 3A). Activities of both Cdh1 and Cdc20 were confirmed by in vitro ubiquitylation of an N-terminal fragment of cyclin B ([^{35}S]cyclin-B^{NT}, Figure 3B). Consistent with the observed stability in oocytes (Figure 2F), destruction box mutant PP6c^{DBM} was not detectably ubiquitylated by APC/C^{Cdh1} (Figure 3A). These data suggest that ubiquitylation of PP6c by APC/C^{Cdh1} targets PP6c for degradation.

Destruction of PP6c Is Critical for Aurora A Activation

Next, we investigated whether PP6c destruction is sufficient to induce meiotic resumption. Since none of the injected MOs depleted PP6c, we injected PP6c antibodies (Ab) into stage-VI oocytes. Injection of PP6c-Ab was not sufficient to induce spontaneous, PG-independent MI entry, but significantly decreased the concentration of PG required to induce GVBD, which was confirmed by immunoblotting for inhibitory Cdk1 phosphorylations (Figures 4A and 4B). We conclude that PP6c inactivation is critical but not sufficient to induce entry into MI, which is in line with the fact that PG triggers multiple parallel pathways which ultimately act in a synergistic manner to fully activate MPF (Karaiskou et al., 2001). Based on these findings, we further speculated that if sustained PP6c activity accounts for the meiotic resumption defect of Cdh1-depleted oocytes, PP6c inactivation in these oocytes should rescue the defect. Indeed, while only 23.5% ± 4.5% of Cdh1-depleted oocytes injected with Ctrl-Ab entered MI within 10 hr after PG treatment, 64.6% ± 4.4% of those co-injected with PP6c-Ab displayed GVBD (Figure 4C). Meiotic resumption was confirmed by

(E) Amino acid alignment of the PP6c destruction box. Consensus residues are shown in green.

(F) Prophase-I arrested oocytes were injected with mRNA encoding myc-tagged full-length destruction box mutant PP6c that was catalytically active (DBM) or inactive (DBM/Ci). At indicated time points after PG exposure, lysates were prepared and immunoblotted.

(G) Three and ten hours after PG stimulation of oocytes from (F), the percentage of oocytes that had undergone GVBD was quantified. n indicates the total number of oocytes analyzed in three independent experiments.

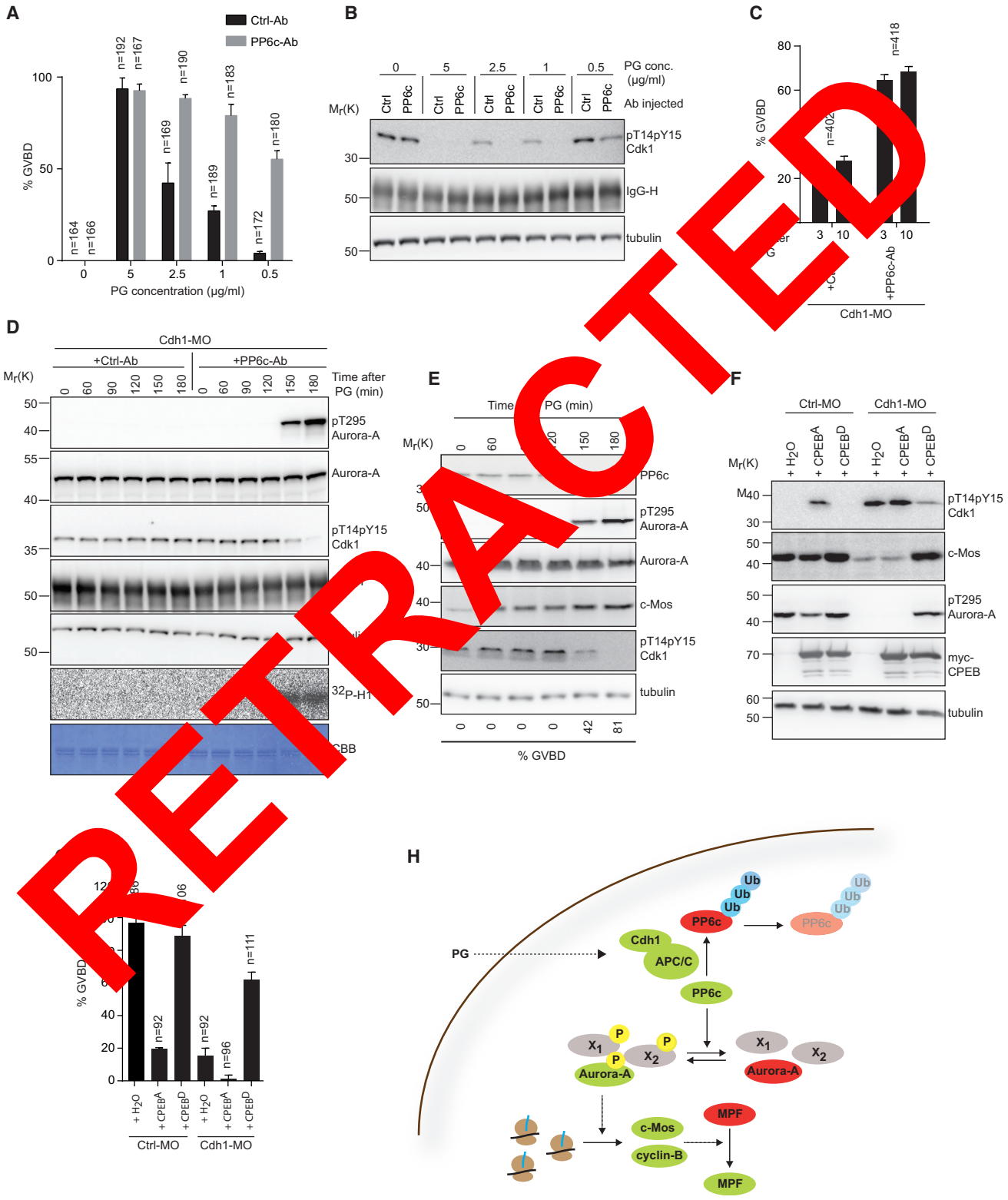

Figure 4. PP6c, Aurora A, and CPEB Form a Critical Axis of Meiotic Resumption

(A) PG titration experiments. Prophase-I arrested oocytes were injected with unspecific immunoglobulin G (IgG) (Ctrl-Ab) or anti-PP6c (PP6c-Ab) and treated with the indicated concentrations of PG. Three hours thereafter, the percentage of oocytes that had undergone GVBD was determined. Error bars indicate SD.

(B) Oocytes from (A) were lysed 3 hr after PG treatment and corresponding extracts immunoblotted as indicated.

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immunoblotting analyses for T14/Y15 phosphorylation of Cdk1 and histone H1 Cdk1 assays (Figure 4D). A recent RNAi-based screen in HeLa cells revealed that PP6c acts as a negative regulator of Aurora A by dephosphorylating it at the regulatory T-loop residue threonine-288 (*Xenopus*: T-295) (Zeng et al., 2010). Autophosphorylation of this residue is necessary for Aurora A kinase activity (Eyers et al., 2003), which, as reported previously, is important for meiotic resumption of immature oocytes (Andresson and Ruderman, 1998). Based on these findings, we speculated that the destruction of PP6c might be required to acquire T-loop phosphorylated Aurora A in response to PG. A key corollary of this model is that the timing of PP6c destruction should coincide with the appearance of T-loop phosphorylated Aurora A. Indeed, analyses of PG-treated oocytes revealed that T-295 phosphorylation coincided with the disappearance of PP6c shortly before GVBD (Figure 4E). To further support the idea that Aurora A is a substrate of PP6c, we investigated the prediction that T-295 phosphorylation should be prevented under conditions interfering with the destruction of PP6c. Indeed, oocytes depleted of Cdh1 failed to accumulate detectable amounts of T-295 phosphorylated Aurora A, and this defect, concomitant with the failure to resume meiosis, was rescued by co-injecting PP6c-Ab (Figure 4F). Thus, the negative correlation of the presence of PP6c and T-loop phosphorylated Aurora A is consistent with the idea that PP6c negatively controls Aurora A activity in *Xenopus* oocytes. The requirement of Aurora A for meiotic resumption has been attributed to its function in promoting the translation of proteins critical for MPF activation (Andresson and Ruderman, 1998; Mendez et al., 2000). A crucial component in this pathway is CPEB (cytoplasmic polyadenylation element binding protein) which, upon phosphorylation at S174 by Aurora A, has been reported to stimulate the polyadenylation and thus translation of the mRNAs encoding c-Mos and B-type cyclins (Mendez et al., 2000). To test the idea that the failure of CPEB phosphorylation contributes to the meiotic resumption defect of Cdh1-depleted oocytes, we co-injected Cdh1 MOs together with H_2O (Ctrl) or mRNA encoding myc-tagged non-phosphorylatable (S174A) CPEB ($CPEB^A$) or phosphomimetic (S174D, $CPEB^D$) CPEB into stage-VI oocytes and quantified GVBD upon PG treatment. Ectopic expression of myc-$CPEB^D$ but not the A mutant restored c-Mos expression, MPF activation (Figure 4F), and consequently meiotic resumption (Figure 4G) in Cdh1-depleted oocytes. These data are consistent with the idea that PG-induced destruction of PP6c is required to allow Aurora A activation, leading to the expression of the M-phase-promoting components c-Mos and B-type cyclins.

DISCUSSION

The selective destruction of cell-cycle proteins via the APC/C is an important regulatory mechanism to ensure irreversibility and unidirectionality of cell-cycle transitions. MPF activity in M-phase promotes the association of the APC with its co-activator Cdc20, resulting in proteasomal destruction of securin and cyclin B at anaphase onset. At low MPF activity the APC/C associates with Cdh1, and during exit from M-phase this interaction mediates the destruction of the remaining cyclin B. During the extended arrest of immature mouse oocytes at prophase-I, APC/C^{Cdh1}-mediated destruction of cyclin B is essential to maintain MPF in its low-kinase state (Holt et al., 2010; Marangos et al., 2007; Reis et al., 2006; Schindler and Schultz, 2009). Similarly, low MPF activity during prolonged prophase-I in budding yeast depends on the destruction of M-phase-promoting proteins via APC/C in complex with the meiosis-specific activator Ama1 (Okaz et al., 2012). Importantly, the transition from prophase-I to metaphase-I requires the inactivation of APC/C^{Ama1} to allow the stabilization of M-phase proteins. In contrast, our study revealed that APC/C^{Cdh1} activity is completely dispensable for prophase-I arrest of immature *Xenopus* oocytes, but essential for MPF activation upon progesterone stimulation. The dispensability of APC/C^{Cdh1} for the prophase-I arrest is consistent with the fact that MPF in immature *Xenopus* oocytes is kept in an inactive pre-MPF state by inhibitory phosphorylations of Cdk1 rather than cyclin B destruction (Dunphy and Newport, 1989; Gautier and Maller, 1991). The search for the relevant APC/C^{Cdh1} substrate identified the catalytic subunit of the serine/threonine phosphatase PP6. PP6 has been implicated in regulating multiple cellular processes including DNA damage repair (Mi et al., 2009), nuclear factor κB signaling (You et al., 2010), and mitotic spindle function (Zeng et al., 2010). Consistent with the identification of PP6 as an Aurora A T-loop phosphatase in proliferating cells (Zeng et al., 2010), we observed that Aurora A T-295 phosphorylation coincided with PP6c destruction shortly before GVBD and was blocked under conditions preventing PP6c destruction. Fully grown, immature *Xenopus* oocytes stockpile dormant mRNAs, and Aurora A has been implicated in promoting oocyte maturation by phosphorylating CPEB, leading to polyadenylation-induced translation of mRNAs encoding c-Mos and B-type cyclins (Sarkissian et al., 2004). In Cdh1-depleted oocytes, expression of phosphomimetic CPEB rescued meiotic resumption, supporting our model (Figure 4H) that the failure to induce Aurora A-mediated expression of M-phase promoting factors accounts for the defect in these oocytes. Recent studies identified additional layers of translational

(C) Prophase-I arrested oocytes were injected with Cdh1-MOs and Ctrl- or PP6c-Ab. At indicated time points after PG stimulation, the ability of oocytes to undergo GVBD was quantified. n indicates the total number of oocytes analysed in three independent experiments.
(D) At indicated time points after PG treatment, extracts of oocytes from (C) were immunoblotted for activating Aurora A phosphorylation (pT295), total Aurora A, and inhibitory Cdk1 phosphorylations. In addition, extracts were supplemented with histone H1 and [γ-^{32}P]ATP. [γ-^{32}P] incorporation was analyzed by autoradiography. IgG heavy-chain (IgG-H) immunoblot shows injected antibodies. CBB, Coomassie brilliant blue staining.
(E) Prophase-I arrested oocytes were treated with PG, and at indicated time points thereafter the percentage of oocytes undergoing GVBD was quantified, and lysates prepared and immunoblotted as indicated.
(F) Prophase-I arrested oocytes were injected with Ctrl- or Cdh1-MOs and subsequently with H_2O or mRNAs encoding myc-tagged $CPEB^A$ (S174A) or $CPEB^D$ (S174D). Ten hours after PG stimulation, oocytes were lysed and immunoblotted as indicated.
(G) The ability of oocytes from (F) to undergo GVBD was quantified 10 hr after PG stimulation.
(H) Model of PG-induced MPF activation.

control in *Xenopus* oocytes involving the sequence-specific mRNA binding proteins Pumilio-2 and Musashi (Charlesworth et al., 2006; Nakahata et al., 2001; Padmanabhan and Richter, 2006). The functional interplay between these factors and CPEB has not been conclusively elucidated, and it is therefore likely that additional PP6c substrates relevant for MI entry will be identified.

The Cdh1 substrate Aurora A remains stable during meiotic resumption of *Xenopus* oocytes, and this observation led to the idea that Cdh1 promotes MI entry in an APC/C-independent manner (Papin et al., 2004). However, as demonstrated by our study, the APC/C co-activator function of Cdh1 is essential to target PP6c for proteolysis. This situation in which certain APC/C substrates such as securin, cyclin B, and Aurora A have to be protected from proteolysis while PP6c is targeted for destruction, is reminiscent of the behavior of APC/C^{Cdh1} during murine female meiosis I, when it targets Cdc20 to ensure proper length of prometaphase while sparing securin and cyclin B (Marangos et al., 2007; Reis et al., 2007; Schindler and Schultz, 2009). Similarly, during the extended prophase-I in budding yeast, APC/C^{Ama1} sees to the destruction of the mitotic transcription factor Ndd1 and M-phase cyclins without targeting securin (Okaz et al., 2012). In mouse oocytes, Hec1 protects cyclin B from APC/C^{Cdh1}-mediated destruction (Gui and Homer, 2013), while Mnd2, the budding yeast homolog of APC15 (Malo et al., 2011), protects securin from ubiquitylation by APC/C in meiosis (Oelschlaegel et al., 2005). Thus, further studies have to clarify exactly how PP6c is selectively destroyed to allow meiotic resumption in *Xenopus* oocytes. In conclusion, our study identifies an unexpected role of APC/C^{Cdh1} and that its activity is critical for MPF activation by targeting PP6c for destruction in response to progesterone stimulation of immature *Xenopus* oocytes.

EXPERIMENTAL PROCEDURES

Handling of Oocytes

Xenopus prophase-I arrested oocytes were surgically removed from adult females, defolliculated using liberase (Cat. #05401127001, Roche) and kept in 5% Leibovitz L-15 medium (supplemented with a penicillin-streptomycin mix) at 18°C before use.

Progesterone Treatment

Ability to mature/prophase-I arrest was determined by the absence of the GV-characteristic white spot. 12–15 hr after treatments, oocytes were treated with PG. To control for oocyte quality, experiments were only considered when >80% of control-treated oocytes underwent GVBD within 3 hr after PG addition. MI entry was determined by the appearance of the GV-characteristic white spot at the animal pole.

Antibodies

See Supplemental Experimental Procedures.

Kinase Assays

Cdk1 and PKA activity were assayed using histone H1 and Kemptide as a substrate, respectively. Oocytes were snap-frozen and lysed in kinase buffer, and centrifuged for 10 min. The supernatant was mixed with H1 or Kemptide and supplemented with [γ-^{32}P]-labeled ATP and incubated for 10 min at 30°C. For H1 kinase assays, samples were analyzed by SDS-PAGE followed by autoradiography. For PKA assays, samples were spotted on p81 Whatman paper and analyzed with a Beckman LS6500 scintillation counter.

In Vitro Ubiquitylation Assays

UBE1 was purchased from Boston Biochem. UbcX and Cdc20/Cdh1 were expressed and purified from *Escherichia coli* and SF9 cells, respectively. Active APC/C was immunopurified from an anaphase HeLa cell lysate. The assay was performed as previously described (Hellmuth et al., 2014).

CPEB Rescue Experiments

Prophase-I arrested oocytes were injected with 128 ng of total mixture of the two Cdh1-MOs or 128 ng of Ctrl-MO. 14–16 hr after injection, MOs, 10 ng of $CPEB^{A}$ or $CPEB^{D}$ mRNAs were injected followed by PG stimulation. GVBD rates were quantified 10 hr after PG stimulation.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures and two figures and can be found with this article online at http://dx.doi.org/10.1016/j.devcel.2015.12.009.

AUTHOR CONTRIBUTIONS

Conceptualization, S.M. and T.U.M.; Methodology, J.M. and P.K.V.; Investigation, S.M.; Writing—Original Draft, S.M., O.S., P.K.V., and T.U.M.; Writing—Review & Editing, S.M., O.S., and T.U.M.; Funding Acquisition, T.U.M.; Supervision, O. S. and T.U.M.

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